Single DNA molecule micromanipulation: a tool to investigate replication enzymes

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In the last ten years, micromanipulation techniques have emerged allowing for the investigation of enzymatic reactions at the single molecule level. We use magnetic tweezers to pull and twist a micron size bead attached to one end of a DNA molecule by biotin/streptavidin while the second end is attached to a glass slide by digoxigenin/antibody. Monitoring the position of the bead with nanometer resolution allows us to record the minute changes caused by a single enzyme interacting with the DNA molecule. In a first example, we shall show how topoisomerases can remove braiding between two molecules or reduce the supercoiling state of a single molecule. In a second example, we demonstrate a molecular motor acting on DNA. We use a hairpin DNA molecule that resembles the "replication fork" used to duplicate DNA in cells. We observe that DNA replication enzymes indeed work on this hairpin. We show, in particular, how the gp41 helicase opens the double helix like a zipper. This experimental assay reveals in real-time how this molecular motor travels along DNA allowing for the accurate investigation of its chemical cycle. This motors is propelled through hydrolyse of ATP. Next we investigate the coupling between the helicase and the primase which synthesises the RNA primer for the Okazaki segment. We show that on priming the primase/ helicase complex may stay bound extruding a single strand DNA loop.